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Research Paper

Poly(Ethylene Oxide)-Modified Poly(β-Amino Ester) Nanoparticles as a pH-Sensitive System for Tumor-Targeted Delivery of Hydrophobic Drugs: Part 2. *In Vivo* Distribution and Tumor Localization Studies

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Purpose. This study was carried out to determine the biodistribution profiles and tumor localization potential of poly(ethylene oxide) (PEO)-modified poly(β-amino ester) (PbAE) as a novel, pH-sensitive biodegradable polymeric nanoparticulate system for tumor-targeted drug delivery.

Methods. The biodistribution studies of PEO-modified PbAE and PEO-modified poly(ε-*caprolactone*) (PCL), a non-pH-sensitive polymer, nanoparticle systems were carried out in normal mice using ^{111}indium-oxine [^{111}In] as a lipophilic radiolabel encapsulated within the polymeric matrix, and the distribution of the nanoparticles was studied in plasma and all the vital organs following intravenous administration. Solid tumors were developed on nude mice using human ovarian carcinoma xenograft (SKOV-3) and the change in concentrations of tritium [^{3}H]-labeled paclitaxel encapsulated in polymeric nanoparticles was examined in blood, tumor mass, and liver.

Results. Study in normal mice with a gamma-emitting isotope [^{111}In] provided a thorough biodistribution analysis of the PEO-modified nanoparticulate carrier systems, whereas ^{3}H-paclitaxel was useful to understand the change in concentration and tumor localization of anticancer compound directly in major sites of distribution. Both PEO-PbAE and PEO-PCL nanoparticles showed long systemic circulating properties by virtue of surface modification with PEO-containing triblock block copolymer (Pluronic®) stabilizer. Although the PCL nanoparticles showed higher uptake by the reticuloendothelial system, the PbAE nanoparticles effectively delivered the encapsulated payload into the tumor mass.

Conclusions. PEO-modified PbAE nanoparticles showed considerable passive tumor targeting potential in early stages of biodistribution via the enhanced permeation and retention (EPR) mechanism. This prompts a detailed biodistribution profiling of the nanocarrier for prolonged periods to provide conclusive evidence for superiority of the delivery system.

KEY WORDS: biodegradable; nanoparticles; noncompartmental pharmacokinetics; pH-sensitive; poly(β-amino ester); poly(ε-caprolactone); tumor targeting.

INTRODUCTION

Poly(β-amino ester)s (PbAE) are synthetic, hydrolytically degradable, biocompatible cationic polymers that are synthesized using parallel synthetic chemistry by addition reaction between primary or secondary diamines and diol-diacrylates (1,2). Because there are a number of different types of diamines and diol-diacrylates, PbAE can be engineered to be water-soluble or -insoluble, having very high or low cationic charge density, different crystallinity, and degradation kinetics to meet the specific requirements of potential use (2,3). Given the polycationic nature of these polymers (due to secondary and tertiary amine groups in the backbone), they are particularly suited for intracellular delivery of DNA and oligonucleotides and allow these systems to remain stable in the cytosol because of their ability to buffer the pH of their surroundings (3,4). Drug delivery systems prepared from PbAE may be used for polynucleotides, proteins, peptides, antigen, and low molecular weight drugs with intracellular targets (1,3,5–7). PbAE degrade under physiological conditions via hydrolysis of their backbone esters to yield small molecular weight bis(β-amino acid) and diol products, which—along with the parent polymer—are significantly less toxic than many other polycations, such as polyethyleneimine and poly(L-lysine) (2,8).

Among United States Food and Drug Administration (FDA)-approved polyesters, poly(ε-caprolactone) (PCL) possesses unique properties such as enhanced biocompatibility, higher hydrophobicity, and neutral biodegradation end products that do not disturb the pH balance of the degradation medium (9–13). Over the years, an array of drug delivery systems has been developed using PCL (14–21).

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Polymeric nanoparticles made from natural and synthetic polymers have drawn major attention because of their higher stability and maneuverability for industrial manufacture, and the opportunity they offer for further surface nanoengineering (22–33). They can be tailor-made to achieve both controlled drug release and disease-specific localization by tuning the polymer characteristics and surface chemistry (23,28–30,34–36). It has been established that nanocarriers can become preferentially concentrated in the tumor mass, inflammatory sites, and at infectious sites by virtue of the enhanced permeability and retention (EPR) effect of the vasculature. As soon as they are accumulated at the target site, hydrophobic biodegradable polymeric nanoparticles can act as local drug depot depending upon the makeup of the carrier, thus providing a source for continuous supply of encapsulated therapeutic compound at the disease site, such as solid tumor (23,28). To prolong the systemic circulation times of the nanoparticles and enhance their passive targeting efficiency, various strategies that involve regulation of particle size, surface charge, and creation of a hydrophilic surface "brush" on the nanomatrices have been employed. Majority of reported strategies employ poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO) chains for surface modification through physical adsorption during particle formation or by covalent linkage to the core-forming polymer [e.g., copolymer of PEG/PEO with poly(lactic acid)] prior to particle formation (19,36–41). More recently, the possibility of introducing PEO-containing block copolymers into the nanoparticles matrix has been achieved by intimate blending of the PEO copolymer with the core-forming polymer prior to nanoparticulate fabrication (42,43). This method of PEO incorporation is expected to lead to partitioning of the PEO chains on the nanoparticle surface upon hydration and better stability of the PEO chains on the nanoparticle surface.

While considering the investigation of any polymeric nanocarrier system for the biodistribution profile, radiolabeling of the polymer backbone is found to be the most favorable and reliable means for subsequent quantification. This chemical conjugation of the radioactive moiety is strongly dependent on the presence of reactive groups in the polymer. However, for hydrophobic polymers such as PbAE and PCL, which do not have any reactive functional groups, the attachment of radiolabel can be difficult. In such cases, the alternative is to encapsulate the radiomarker within the polymeric matrix. Gamma ray emitting labels are usually preferred for evaluating the biodistribution because of higher sensitivity and ease of sample preparation and processing. In certain cases, the bioactive compounds are labeled with beta-emitting species (like tritium) and become helpful in tracing the pathway of distribution of the pharmacologically active compound *in vivo*.

In the present study, we chose indium-111 (^{111}In) as the label for the nanoparticles mainly because of its shorter half-life (2.8 days) and its availability in the lipophilic form (^{111}In oxyquinoline or ^{111}In oxine). For tumor uptake and concentration studies, we selected paclitaxel as the most successful cytotoxic agent against solid tumors, which was readily available in tritiated form. A systematic *in vitro* investigation of the nanoparticles in tumor cells formed the basis for the current *in vivo* studies (44).

MATERIALS AND METHODS

Materials

A representative hydrophobic PbAE (MW ~10,000) was synthesized by the addition reaction of 4,4′-trimethyldipiperidine with 1,4-butanediol diacrylate in dimethylformamide for 48 h at 50°C, and purified according to the protocol described earlier (2,5). PCL, with a number average molecular weight of 14,800 Da (as verified by gel permeation chromatography), was purchased from Polysciences Inc. (Warrington, PA, USA). National Formulary grade Pluronic® F-108 was kindly provided by the Performance Chemical Division of BASF Corporation (Parsipanny, NJ, USA). Paclitaxel was purchased from LC Laboratories (Woburn, MA, USA) and tritiated (^{3}H) paclitaxel with an activity of 3.2 Ci/mmol was purchased from Moravek Biochemicals (Brea, CA, USA). Paclitaxel solution, available as a commercial injection in Cremophore EL ethanol (Onxol®; 50:50 mixture) was purchased from Ivax Pharmaceuticals (Miami, FL, USA). Indium (^{111}In) oxyquinoline solution was purchased from Amersham Health (Arlington Heights, IL, USA) and had an activity of 1 mCi/mL at calibration time. SKOV-3, human ovarian adenocarcinoma cells, were kindly supplied by Dr. Michael Seiden's laboratory at Massachusetts General Hospital (Cambridge, MA, USA). All the other chemicals and reagents were of analytical grade and were used as supplied. Deionized distilled water (NanoPure II, Dubuque, IA, USA) was used for all aqueous preparations.

Preparation and Characterization of Radiolabeled Polymeric Nanoparticles

^{111}In-Labeled Polymeric Nanoparticles

^{111}In oxy-quinoline (or ^{111}In oxine) is commercially available as an aqueous solution for injection. Prior to incorporation into the nanoparticles, it is necessary to extract ^{111}In oxine into an organic phase (chloroform). Two milliliters of chloroform was gently mixed with 250 μl of the ^{111}In oxine for 10 min and allowed to separate. The organic layer was carefully pipetted into two clean glass vials in equal volumes. Contents of one vial were used to dissolve known quantities of PbAE and Pluronic® F-108 (25+6.5 mg) and the other was used to dissolve PCL and Pluronic® F-108 (25+6.5 mg). The organic solvent was evaporated under a gentle stream of dry nitrogen. Absolute ethanol and acetone (2.5 mL each) were used to redissolve the residues of PbAE and PCL blends with Pluronic® F-108 and ^{111}In-oxine, respectively. This organic solution was introduced into precooled (<15°C) purified water (12.5 mL, pH adjusted to 7.0) maintained under vigorous magnetic stirring. Gentle stirring was continued for 5 h to evaporate majority of the organic solvent from the bulk. The nanoparticles were collected by centrifugation (10,000 rpm for 20 min), washed once with 5 mL of water (pH 7.0), and again collected by centrifugation. The final suspension was made in known volume of sterile phosphate-buffered saline (PBS, pH 7.4) and the formulation was ready to be injected.

^{3}H-Paclitaxel Encapsulated Nanoparticles

For preparing drug-loaded nanoparticles, cold paclitaxel (3.5 mg) was dissolved along with the polymers (blends of PbAE-Pluronic® F-108 and PCL-Pluronic® F108) in organic phase (acetone or ethanol) before introduction into aqueous medium. A suitable quantity (15 μCi) of ^{3}H-labeled paclitaxel was incorporated in the organic phase to obtain tritiated nanoparticles, and the products were collected and purified as described in the previous section.

PEO-modified PbAE and PCL nanoparticles without any radioactive payload were prepared and diluted suitably in deionized distilled water (pH ~7.0). Particle size was determined by Coulter® N4-Plus submicron particle sizer (Coulter Corporation, Miami, FL, USA) at multiple scattering angle detection. For zeta potential measurements, a diluted aqueous suspension of nanoparticles was mounted in a 90Plus particle sizer/zetasizer (Brookhaven Instruments, Brook-haven, NY, USA) and mean zeta potential was computed using Smoluchowski equation.

In Vivo Experiments

The experimental protocol involving usage of radioactive materials in animals was approved by the Institutional Animal Care and Use Committee and the Office of Environmental Health and Safety at Northeastern University. Female athymic mice (Nu/Nu strain), 4–6 weeks old, weighing about 25 gm were purchased from Charles River Laboratories (Cambridge, MA, USA) and were housed in polycarbonate cages having free access to sterilized rodent pellet diet and water. The animals were allowed to acclimate for at least 48 h prior to any experiments.

Biodistribution Studies of ^{111}In-Labeled Polymeric Nanoparticles

Nanoparticle biodistribution studies were carried out in female Nu/Nu without any tumor load. We used this particular species because Nu/Nu mice with human ovarian cancer xenograft were subsequently used for paclitaxel accumulation studies. Eight Nu/Nu mice without any xenografted tumors were used per time-point (0.5, 1, 6, 12, and 24 h) and were accordingly divided into two treatment groups. One group of mice received ^{111}In-labeled PEO-modified PbAE nanoparticles and the second group received ^{111}In-labeled PEO-modified PCL nanoparticles. The formulations were suspended in PBS and contained approximately 10 μCi of ^{111}In dose per mouse. Injections were given through the tail vein as a slow bolus. At periodic time intervals, blood was collected through sino-arbital vein puncture under light anesthesia (with isoflurane). The animals were then sacrificed by cervical dislocation and the vital organs were harvested, washed with sterile PBS, and collected in preweighed glass tubes. Radioactivity counts was obtained using a gamma counter (Wallac-Coulter, Miami, FL, USA) and the counts per minute (cpm) were converted to Ci units and reported as percentage activity recovered per gram of fluid or tissue.

Tumor Concentrations of Paclitaxel in SKOV-3 Bearing Mice

The SKOV-3 human ovarian carcinoma cells were grown in culture flasks containing RPMI 1640® medium modified with fetal bovine serum and antibiotics. They were harvested and resuspended in serum-free medium (SFM) before injection into nude mice. Approximately, 2 million tumor cells suspended in 100 μl of SFM were injected subcutaneously into the dorsal side of mice under light isoflurane anesthesia. Solid tumors developed within 4 weeks posttumor inoculation and as soon as tumor volume reached approximately 200 mm^{3}, the animals were chosen for experimental treatment.

Four mice were used per time-point (1, 6, 12, 24, and 48 h) and there were three treatment groups—control (paclitaxel in solution), paclitaxel in PEO-modified PbAE nanoparticles, and paclitaxel in PEO-modified PCL nanoparticles. The formulations were diluted and suspended in sterile PBS and contained approximately 1 μCi of tritium dose per mouse. The injections were given through the tail vein as slow bolus. At periodic time intervals, blood was collected through sino-arbital vein puncture under light anesthesia (with isoflurane). The animals were then sacrificed by cervical dislocation, and liver and tumor were harvested. The tissues were washed with PBS and collected in preweighed glass tubes. A 10% (w/v) homogenate was prepared in water and 1.5 mL each was added to a scintillation vial. All tissues and fluids (blood) were digested with Scintigest® fluid (from Fisher Scientific, Pittsburgh, PA, USA; 1 mL, incuba-tion for 2 h at 50°C) and decolorized with hydrogen peroxide (200 μl of 30% solution, incubation for 30 min at 50°C). Then, scintillation cocktail (ScintiSafe® Econo 1, 10 mL; Fisher Scientific) was added and the sample was allowed to quench for 2 h in dark before measurement was carried out in a liquid scintillation analyzer (TriCarb 1600TR; Packard Instrument Co., Meriden, CT). The unit cpm was converted into μCi using appropriate calibration curves.

Noncompartmental Pharmacokinetic Analysis

Noncompartmental pharmacokinetic analysis was performed on the raw data expressing plasma concentration *vs.* time profiles for the nanocarriers and the parameters were calculated using Win-Nonlin® software (Pharsight Corp, Mountain View, CA, USA).

RESULTS AND DISCUSSION

Polymeric Nanoparticle Preparation and Characterization

Spherical nanoparticles having smooth surface and distinct boundary were obtained by solvent diffusion method. Nanoparticles were instantaneously formed with the newly formed nanosurface being sterically stabilized through the PEO chains of the polymeric stabilizer (Pluronic®). Particles obtained with PbAE were in the range of 100–150 nm with a mean diameter of 113 nm. PbAE nanoparticles without any payload had a surface charge of 46.8 mV (in purified water) and upon drug loading the value was slightly reduced to 39.4

mV. We optimized the formulations at a paclitaxel loading of 20% by weight and achieved an entrapment efficiency of 95% through regulation of the organic-to-aqueous phase ratios and polymer concentrations. The PCL nanoparticles had a mean diameter of about 200 nm and the surface charge was 30.8 mV with 20% by weight paclitaxel loading.

The higher hydrophobicity of PCL and PbAE plays a key role in the efficient surface modification strategy using Pluronic® triblock copolymers having an ABA structure of PEO and poly(propylene oxide) (PPO) segments. Evidence for surface modifications of PCL and PbAE nanoparticles with Pluronics and the stability of Pluronic layer has been reported in our earlier reports (17,44). The surface modification is purely dependent on the hydrophobic interactions between the center-block (PPO) of the stabilizing surfactant and the polymeric core (PCL or PbAE nanocarrier). In the present study, we propose to use Pluronic® F108 having 56 residues of propylene oxide (PO) and 122 residues of ethylene oxide (EO). By blending PCL and PbAE and the Pluronic® copolymers in an appropriate proportion, the hydrophilic PEO side-arms remain in the mobile state as they extend outwards from the particle surface and provide stability to the particle suspension by a repulsion effect through a steric mechanism of stabilization involving both enthalpic and entropic contributions. The end result of such an assembly is a stable, slow-eroding (PEO-PCL) or faster-eroding (PEO-PbAE) colloidal system that is less phagocyte-prone (hence long circulating). This type of surface modifi-cation strategy is sufficiently versatile and can be applied to a number of other hydrophobic polymeric systems. Also, there are over 15 different types of Pluronic® copolymers available containing different chain lengths of PEO and PPO and, as such, the nanoparticle surface can be specifically engineered for different applications.

Preparation of Radiolabeled Polymeric Nanoparticles

The solvent displacement method is convenient for preparing hydrophobic nanoparticles reproducibly and with well-defined and controlled properties. This method is very useful for encapsulation of lipophilic drugs, such as paclitaxel. The encapsulation efficiency in such situations is generally very high (>95%). When there are no chemically reactive functional groups present in the polymer for exploring labeling through conjugation, encapsulation of the radiolabel within the particles is the only viable option. Such methods have been extensively reported in the past for lipophilic drugs that have a radioactive marker conjugated to them. The labeled drug is distributed in the nanomatrix and the delivery system as a whole represents a monolithic assembly. However, there are very few reports for a similar system where a lipophilic radiolabel derivative has been encapsulated. One reported example is that of polymeric nanocapsules with the lipophilic radioactive compound dissolved in an oily core (45,46).

^{111}In is a popular radioactive marker used in diagnostic medicine for many reasons: (1) it has a clinically appreciable half-life (2.8 days), (2) it is available in the form of aqueous injections (^{111}In chloride and ^{111}In oxine), (3) it is stable in the systemic environment, (4) and it requires a simple process to expose tissues and fluids before counting. The lipophilic derivative (^{111}In oxine) was previously reported for studying the biodistribution of biodegradable nanocapsules made from poly(D,L-lactic-*co*-glycolic acid) (PLGA) and PCL (45,46). ^{111}In oxine was extracted into olive oil, which formed the core of the nanocapsules that were prepared by solvent evaporation method. As we wanted to utilize the solvent displacement method for the preparation of the nanoparticles (and not nanocapsules), we had to suitably modify the technique. The extraction step was carried out using chloroform (a lipophilic solvent having excellent solubility for both polymers used) with an efficiency of 75%. To prevent the loss of the extracted radioactive compound during evaporation of chloroform, the polymer blends (PbAE + Pluronic® or PCL + Pluronic®) were first dissolved into the ^{111}In oxine containing chloroform, followed by exposure to gentle stream of nitrogen to obtain a sticky residue of the radioactive–polymer blend. If one chooses to follow solvent evaporation technique for the preparation of nanoparticles, the organic solution of the polymer blends with ^{111}In oxine can be directly employed. However, as we chose to follow a well-standardized solvent displacement technique, the solvent for polymer had to be changed from water-immiscible organic solvent (chloroform) to a water-miscible (hence water-displaceable) solvent such as absolute ethanol (for PbAE) or acetone (for PCL). The labeling efficiency was once again excellent (about 90% of the radiolabel being incorporated) as expected for a lipophilic agent. The advantage in the current situation was that the radiolabel was distributed within the entire polymeric nanomatrix rather than the surface. For those systems utilizing surface-bound radiolabel, one is concerned about the stability and subsequent interpretation of the results.

In the case of ^{3}H-labeled paclitaxel, the radiolabel was in the form of chemical conjugation with the cytotoxic agent and was incorporated within the nanoparticles as the drug itself to form a monolithic matrix-based nanocarrier system. Incorporation efficiency was about 95%. In both cases, the labeling step was found to be fast, easy, and reproducible, and binding was expected to be stable within the systemic environment after intravenous administration.

Biodistribution Studies of ^{111}In-Labeled Nanoparticles

As the first step for studying the overall biodistribution pattern of the nanoparticles, we chose to use blank PEO-modified PbAE and PCL nanoparticles with an incorporated radiolabel in normal mice. A gamma emitting label is desirable for the purpose as it would drastically reduce the processing and estimation times because of the large volume of tissue samples generated. Additionally, the developed system may also be applied in gamma imaging applications for diagnostic purposes.

The results obtained for the biodistribution profile of ^{111}In-labeled PEO-modified PbAE and PCL nanoparticles are presented in Figs. 1 and 2 and Table I. The two polymers showed different distribution profiles and kinetics depending upon their properties. In general, the both PEO-modified PCL (PEO-PCL) nanoparticles and PEO-modified PbAE (PEO-PbAE) nanoparticles showed higher tendency for

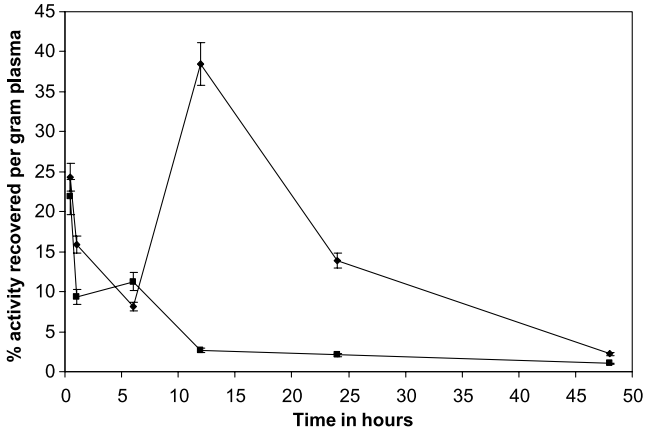

Fig. 1. Plasma concentrations *vs.* time profiles of ^{111}indium oxine-labeled poly(ethylene oxide)-modified poly(ε-caprolactone) (PCL) nanoparticles (■) and poly(ethylene oxide)-modified poly(β-amino ester) (PbAE) (♦) nanoparticles in mice.

accumulation in highly perfused organs such as liver, spleen, and lungs during the initial phase, and in kidney at the later stages of biodistribution.

During the initial phase of distribution, PEO-PbAE nanoparticles tend to be trapped within the microvasculature of the lungs (Fig. 2B). Given the proof that in our study, the size as well as the zeta potential of the two types of

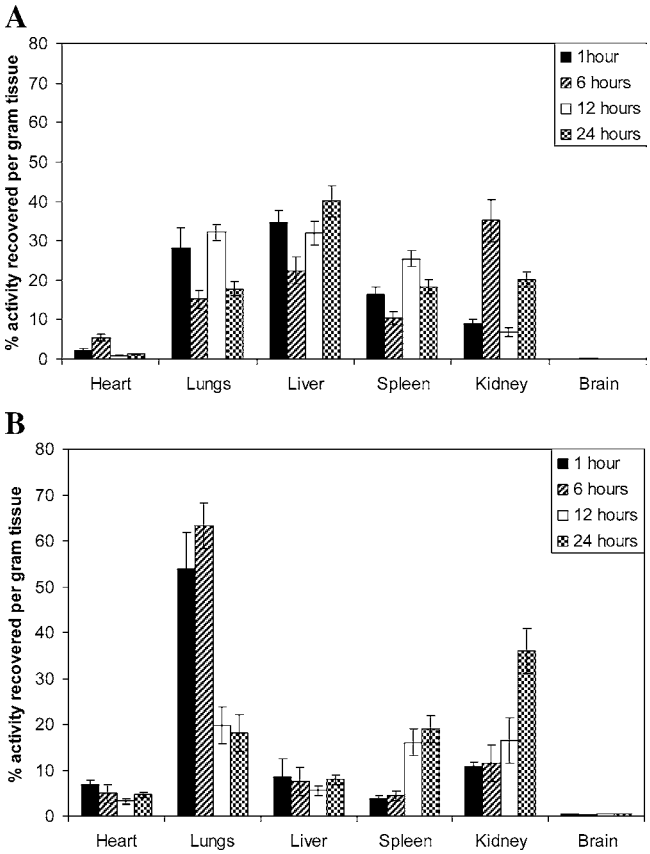

Fig. 2. Biodistribution profiles of ^{111}indium oxine-labeled poly (ethylene oxide)-modified poly(ε-caprolactone) (PCL) nanoparticles (A) and poly(ethylene oxide)-modified poly(β-amino ester) (PbAE) nanoparticles (B) in mice.

Table I. Noncompartmental Pharmacokinetic Parameters from Plasma Concentrations *vs.* Time Profiles after Intravenous Administration of ^{111}In-Labeled PEO-Modified PbAE and PCL Nanoparticles

Pharmacokinetic parameter	PEO-PbAE nanoparticles	PEO-PCL nanoparticles
Half-life (h)	20.55 ± 1.73[a]	25.3 ± 0.34
Mean residence time (h)	21.03 ± 0.69	24.0 ± 0.23
Volume of distribution [% (%/g/g)]	2.49 ± 0.43	10.2 ± 0.89
Clearance [%h (%/g)/g]	0.12 ± 0.06	0.43 ± 0.04
Maximum concentration (%/g)	37.5 ± 1.25	51.7 ± 0.83
Area under the curve (h %/g)	849.4 ± 48.98	236.0 ± 18.40

[a] Values are expressed as mean ± SD (n = 4).

nanoparticles were comparable, there is a possibility of PEO-PbAE nanoparticles undergoing aggregation upon introduction into the systemic circulation. This could be induced by interactions with the components of the plasma or by gradual displacement of the hydrophilic PEO chains from the nanoparticle surfaces. Similarly, for PEO-PCL nanoparticles, the profile represents a rapid localization of a significant percentage of the dose within the liver and spleen through RES uptake (Fig. 2A). Previous reports on ^{111}In oxine injection have proven that upon intravenous administration, it is quickly bound to plasma protein-transferrin (47). Subsequently, the marker is taken up by the liver cells although the transferrin receptor and a progressive elimination of the marker occurs. For the nanoparticle-encapsulated indium, meanwhile, the elimination proceeds through the uptake of the particle by the liver through a process mediated by opsonization. After the internalization of the nanoparticles, indium oxine is released and converted to its hydrophilic form, which may then be eliminated through the kidneys.

There was no significant difference between the two carriers with respect to elimination half-life, mean residence time, or maximum concentrations in the blood (Table I). The plasma half-life of unmodified (without PEO coating) polymeric nanoparticles usually ranges from 1 to 10 min (23), but PEO-surface modification can significantly extend the half-life, as we have observed in the present case. About 4 times higher volume of distribution was observed for PEO-PCL nanoparticles because of their extensive extravascular distribution in well-perfused tissues such as liver, lungs, and kidney (Fig. 2A). For PEO-PbAE nanoparticles, an initial localization was found in lungs, which dropped quickly—resulting in simultaneous increase in counts for blood and kidney (Fig. 2B). An increase in ^{111}In concentrations in the kidney suggest that there could be a disintegration/dissolution of the nanoparticles occurring at its residence in lung capillary bed—probably as a result of the reduced systemic pH, thus releasing a portion of the radioactive payload.

About 3.5 times higher AUC for PEO-PbAE nanoparticles in the systemic circulation suggest an enhanced performance of the nanocarrier with respect to the systemic presence/bioavailability and a balanced distribution compared to PEO-PCL nanoparticles. A higher magnitude in AUC may also indicate a quicker release of the contents of the PbAE nanomatrix compared to the PCL.

Tumor Uptake Studies of Paclitaxel-Containing Nanoparticles

Majority of the injected dose (approx. 60–80%) in the case of nanoparticles reached the liver compared to the control (Onxol® injection). The liver uptake was significantly higher for PEO-PCL nanoparticles. PEO-PCL nanoparticles and the control formulation showed prolonged presence in blood for two different reasons (Fig. 3). PEO modification leads to significantly higher circulation times for polymeric nanoparticles because of a decrease in the degree of interactions with the plasma proteins and hence reduced clearance from the systemic circulation. Being highly hydrophobic, paclitaxel is an extensively protein-bound drug (>99%) and hence when administered in solution form, it exhibited pronounced blood concentrations during the entire study period. PEO-PbAE nanoparticles were cleared from the systemic compartment with a corresponding increase in tumor concentration (Figs. 3 and 4). The trend observed in Fig. 1 indicates the long circulating property for PEO-modified nanoparticles. At the same time, in the presence of a compromised (or fenestrated) capillary network (as in a solid tumor), they seem to get cleared more efficiently from the general circulation than PEO-PCL nanoparticles (Fig. 3) and show a higher efficiency to get accumulated within the tumor mass predominantly by virtue of EPR mechanism (Fig. 4). It is clear from the tumor concentrations of the drug that both nanoparticle formulations can deliver a significantly higher concentration of the drug into the tumor than the solution form. At 1 h post-administration, for instance, there was a 5.2-fold higher concentration of paclitaxel in the tumor when delivered in PEO-PbAE nanocarriers and 2.2-fold higher concentrations with PEO-PCL nanocarriers as compared to the aqueous solution. Results were even more pronounced at 5 h post-administration, with almost 23-fold higher paclitaxel concentrations for PEO-PbAE and 8.7-fold higher concentrations for PEO-PCL relative to the aqueous solution formulation.

PEO-PCL nanocarriers clearly exhibited long-circulating properties in the plasma as shown by the half-life and mean

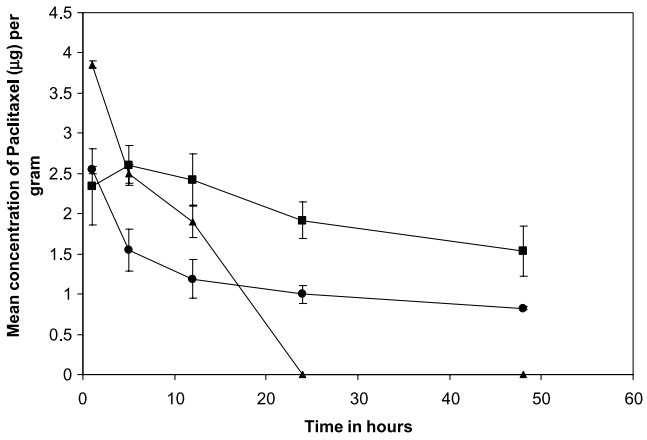

Fig. 3. Plasma concentrations *vs.* time profiles of tritiated [^{3}H]paclitaxel upon intravenous administration to SKOV-3 human ovarian adenocarcinoma-bearing nude mice. Paclitaxel was administered in aqueous solution as control (●), in poly(ethylene oxide)-modified poly(ε-caprolactone) nanoparticles (■), and poly(ethylene oxide)-modified poly(β-amino ester) (PbAE) nanoparticles (▲).

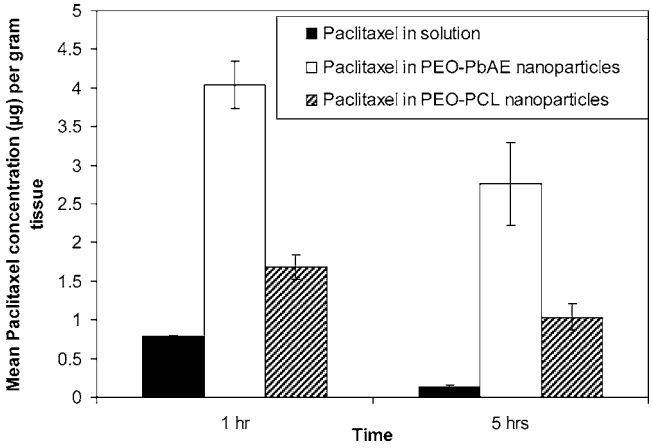

Fig. 4. Tumor concentrations as a function of time of uptake of tritiated [^{3}H]paclitaxel upon intravenous administration to SKOV-3 human ovarian adenocarcinoma-bearing nude mice. Paclitaxel was administered intravenously in aqueous solution as control, in poly(ethylene oxide)-modified poly(ε-caprolactone) nanoparticles (PCL), and poly(ethylene oxide)-modified poly(β-amino ester) (PbAE) nanoparticles.

residence time values of 25.3 and 24.0 h, respectively. The long residence time of PEO-PCL nanocarriers in the systemic circulation is evident by an almost 8-fold lower total body clearance value [0.43 *vs.* 3.45 (%h (%/g)/g)] as compared to the free drug. The pharmacokinetic results clearly show that PEO-PCL and PEO-PbAE had long-circulating properties in the systemic circulation and could be targeted to the tumor mass by the EPR effect.

In our earlier studies on PEO-PCL nanoparticles encapsulated with ^{3}H-labeled tamoxifen, similar results were obtained upon intravenous administration in MDA-MB-231 human breast adenocarcinoma-bearing nude mice (17). It is likely because of its pH-sensitive behavior (increased solubility at pH < 6.5) that the PbAE-based nanocarriers are dissolving rapidly and providing the additional benefit of triggered drug release within the low-pH environment existing within tumor mass, leading to high localized drug concentrations (44).

CONCLUSIONS

PEO-modified PbAE and PCL nanoparticles can be successfully prepared by blending the polymers with Pluronic® F-108. The PPO central block gets anchored onto the nanoparticle surface more strongly than mere physical adsorption, leaving the hydrophilic PEO chains mobile on surface leading to steric stabilization of the nanocarrier. Both types of nanoparticles can be radiolabeled during the manufacturing step through the physical entrapment of a lipophilic marker and can be used for determination of biodistribution profile after intravenous administration. PEO-surface modification rendered both nanoparticulate carriers long circulating (compared to unmodified nanocarriers) as evidenced by increased half-lives. PEO-modified PbAE nanoparticles were highly efficient in delivering the cytotoxic cargo to the tumor site through EPR localization, whereas PEO-modified PCL nanoparticles showed high extravascular distribution. PbAE nanoparticles, being pH-

sensitive, could deliver the entire payload more rapidly compared to PCL-based system and hence nanoparticles can be highly efficient carrier systems for cytotoxic agents to achieve rapid tumoricidal action.

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